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Cytomegalovirus Antibody Avidity in Allogeneic Bone Marrow Recipients: Evidence for Primary or Secondary Humoral Responses Depending on Donor Immune Status

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The reconstitution of the human cytomegalovirus (CMV) antibody response in CMV seropositive bone marrow transplant patients was investigated by comparing 11 patients whose donors were CMV seropositive with 8 whose donors were CMV seronegative. Evidence for primary or secondary responses to CMV was sought by determining IgG antibody avidity using an avidity index method, and antibody titre over a period of up to 3 years after transplant. For the patients whose donors were CMV seropositive, the results showed the characteristics of a secondary response, i.e., rising antibody titres of high avidity immediately after transplant. In contrast, the patients with CMV seronegative donors showed evidence of a primary antibody response usually occurring at about 250 days after transplant, i.e., rising antibody levels initially of low avidity maturing to high avidity over the following 100 to 200 days.

It is concluded that a secondary response and hence transfer of humoral immunity had occurred in those patients whose donor was CMV seropositive, whereas a delayed primary response occurred in those patients whose donor was CMV seronegative.

KEY WORDS: CMV, bone marrow transplant, immune reconstitution, B cell, avidity index, IgG antibody titre

INTRODUCTION

Human cytomegalovirus (CMV), in common with the other human herpesviruses, persists for life after primary infection and the infected individual is therefore at risk of recurrent infection and also disease if immunosuppressed. Indeed, CMV is an important cause of morbidity and mortality in CMV seropositive allogeneic bone marrow recipients [Winston et al., 1990] in whom pretransplant conditioning with irradiation and chemotherapy ablates both humoral and cellular immunity which only recover slowly as donor cells engraft. Although it is well established that recovery of cytotoxic T cell function is very important for protection against severe CMV infection in such patients [Quinnan et al., 1982], the role and course of B cell reconstitution, and hence of production of antibody specific for CMV, are less clear.

Considering the general phenomenon of B cell reconstitution following bone marrow transplantation, after an initial leucopenia the host is soon repopulated with donor lymphocytes but full restoration of humoral immunity lags behind because recapitulation of immune ontogeny must occur; engrafted donor B cells only commence immunoglobulin production slowly and primary antibody responses to neoantigens do not return to normal for months to years after transplant [Storek and Saxon, 1992]. However, when memory B and T lymphocytes for particular viral antigens are already present in donor marrow, such cells might allow rapid reconstitution of the humoral response to the antigens in question. Such a speedy reacquisition of humoral immunity will be much more likely if the relevant virus persists in the recipient and hence viral antigens are available for presentation to the repopulating donor memory lymphocytes. This situation, which occurs when CMV seropositive patients receive bone marrow from a donor who is also CMV seropositive, has been investigated in two recent studies [Boland et al., 1992; Roy et al., 1993]. In both studies it was found that if the donor was CMV seropositive, CMV antibody titres were greater than if the donor was CMV seronegative. The inference from this work is that a secondary response with rising antibody titres to CMV occurred when donor memory cells specific for CMV were present. The follow-up period for both studies was short (up to six months) and there was no indication as to the eventual outcome in those recipients whose donors were CMV seronegative. How-

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TABLE I. Comparison of Mean CMV Antibody Titre in Seropositive Patients Given Bone Marrow From Seropositive or From Seronegative Donors

Time after transplant (months)	Patients with seropositive donors (Nos. 1–11) Mean antibody titre[a]	95% confidence limits	Patients with seronegative donors (Nos. 12–19) Mean antibody titre[b]	95% confidence limits	P
0 (pretransplant)	7.6*	[6.6–8.6]	6.9	[4.0–9.8]	>0.5
6	9.4	[7.3–11.4]	6.1	[4.3–7.9]	<0.02
12	9.7*	[7.9–11.5]	5.6	[3.6–7.6]	<0.01

[a]Antibody titre is expressed as $\log_2$; number of sera tested was 11 in each case.
[b]Antibody titre is expressed as $\log_2$; number of sera tested was 8 at 0 and 6 months, and 7 at 12 months.
*The difference between these two means is significant; $P < 0.05$.

ever, it seems likely that antibody levels would decrease initially as recipient B cells and antibody slowly disappear, and then rise as repopulating virgin lymphocytes originating from the donor finally achieve immunocompetence and mount a delayed primary immune response to CMV [Storek and Saxon, 1992].

In order to confirm such a primary response to CMV, IgM and IgG seroconversion should be documented, but this is not possible after allogeneic bone marrow transplantation because of persisting antibody in the recipient. Moreover, anti-CMV IgM may be produced by immunosuppressed patients undergoing either primary or secondary responses [Pass et al., 1983]. These difficulties would be overcome by means of a CMV IgG antibody avidity test since the avidity of antibody increases progressively with time after first exposure of the immune system to an immunogen [Eisen and Siskind, 1964], thus indicating a primary response, whereas a secondary response is of high avidity throughout.

A suitable CMV IgG avidity enzyme-linked immunosorbent assay (ELISA) has been developed for routine diagnostic use [Blackburn et al., 1991] and further validated for the investigation of CMV infection in solid organ transplant recipients in our laboratory [Lutz et al., 1994]. The present study investigated both CMV IgG antibody titre and avidity in 19 CMV seropositive patients with chronic myeloid leukaemia who received an allogeneic bone marrow transplant from a sibling donor who was either CMV antibody positive or negative.

MATERIALS AND METHODS

Patients

All patients studied had chronic myeloid leukaemia and received a bone marrow transplant from a sibling donor during the period 1987 to 1991 [Marks et al., 1992]; the transplant protocol did not involve T-cell depletion. Patients were included in the study only if a sufficient number of stored sera were available (see below); no other selection criteria were applied.

Patients and donors CMV seropositive before transplant (Nos. 1 to 11). Four male and 7 female patients (mean age 34 years at time of transplant; range 24–46) and their 11 corresponding sibling donors (mean age 34 years; range 27–50) were investigated.

Patients CMV seropositive before transplant with seronegative donors (Nos. 12 to 19). Five male and 3 female patients (mean age 30 years at time of transplant; range 14–43) and their 8 corresponding sibling donors (mean age 37 years; range 5–53) were investigated.

Sera

A blood sample was taken from each patient before transplantation and sent to the Diagnostic Virology Laboratory, Royal Postgraduate Medical School, London, for routine virus investigation. Thereafter serial samples were sent over a period of at least 1 year and in 2 cases for as long as 3 years. Serum from each patient's donor was also tested.

Quantification of CMV Antibodies

A latex agglutination test for CMV antibodies (CMV scan, Becton Dickinson) was performed according to the manufacturer's instructions. The antibody titre was determined by testing serial doubling dilutions of patients' sera. The result was expressed as $\log_2$ of the inverse of the lowest dilution in which antibody was detected. The latex test was also used to determine whether donors were CMV seropositive or seronegative [Gray et al., 1987].

Anti-CMV IgG Avidity Test

A commercially available CMV IgG ELISA (CMV stat, Bio Whittaker UK Ltd) was used with a modified procedure; for full details see Lutz et al. [1994]. Briefly, for each sample, diluted sera were added to four antigen-coated wells and incubated for 15 minutes at room temperature. All wells were then washed twice with phosphate buffered saline containing 0.05% Tween 20 (PBS-Tween) following which 2 of the 4 wells were filled with PBS-Tween and 2 with PBS containing 8M urea. After 5 minutes, all wells were washed once more with PBS-Tween and the test was completed according to the manufacturer's instructions. The absorbance at 550nm (OD_{550}) for each well was read using a Titertek Multiscan Plate Reader which gives linear readings up to an optical density of 2 and non-linear readings between 2 and 3. The amount of CMV IgG present in each serum was

expressed as the mean OD_{550} from the 2 wells not exposed to urea. The antibody avidity index was calculated as the mean OD_{550} from the urea-washed wells divided by the mean OD_{550} from the wells not exposed to urea, expressed as a percentage. Sera known to contain high and low avidity anti-CMV IgG [Lutz et al., 1994] were included in each test as controls. Since estimation of the avidity index may be unreliable where the antibody concentration is low, the index was not recorded in cases where the OD_{550} was less than 0.75 in the absence of urea.

Statistics

The 95% confidence limits for a mean avidity index or antibody titre were calculated from the standard error of the mean and are shown in each case in square brackets after the mean. The unpaired Student's t-test was used to estimate the significance of the difference between two means.

RESULTS

The Effect of Donor CMV Antibody Status on Patients' Antibody Titres After Transplant

Table I shows a comparison of the mean antibody titres, as measured quantitatively by the latex test, in patients given bone marrow from seropositive (Nos. 1–11) or seronegative donors (Nos. 12–19) during the first year after transplant. Although the mean antibody titres for the two groups were not significantly different prior to transplant, mean antibody titres rose significantly in those patients whose donor was CMV seropositive whereas antibody levels remained stable and significantly lower in those patients whose donor was CMV seronegative. The mean antibody titre for the donors to patients 1–11 was 6.6 [5.1–8.2] which is not significantly different from the mean antibody titre for either group of patients prior to transplant.

The difference in antibody production between the two groups of patients after transplant was also confirmed qualitatively by the results of the CMV IgG ELISA given in units of OD_{550} for individual patients 1–11 and 12–19 covering a period of at least a year and in 2 cases up to 3 years (mean length of follow up 584 and 613 days, Figures 1 and 2, respectively). Figure 1 shows the data for each patient whose donor was CMV seropositive (Nos. 1–11). For 9 of these patients the amount of CMV IgG as measured by the ELISA was high before transplant and not quantifiable (OD_{550} greater than 2.0), and remained so throughout the period of study. In the remaining 2 cases (Nos. 2 and 4) the amount of antibody clearly increased from a starting OD_{550} of 1.5–2.0 to a final value of 2.5–3.0. Overall in this group of patients antibody levels were high and in no case was the OD_{550} after transplant below 1.9.

In contrast, for patients whose donor was CMV seronegative (Nos. 12–19), Figure 2 shows that the usual pattern is for a marked drop in the amount of antibody soon after transplant of at least 0.5 and up to 1.5 OD_{550}, reaching the lowest value within 250 days and followed by a rise (patients 12, 13, 15, 16, and 18). Some variation from this occurred: in patient 17 there was a marked

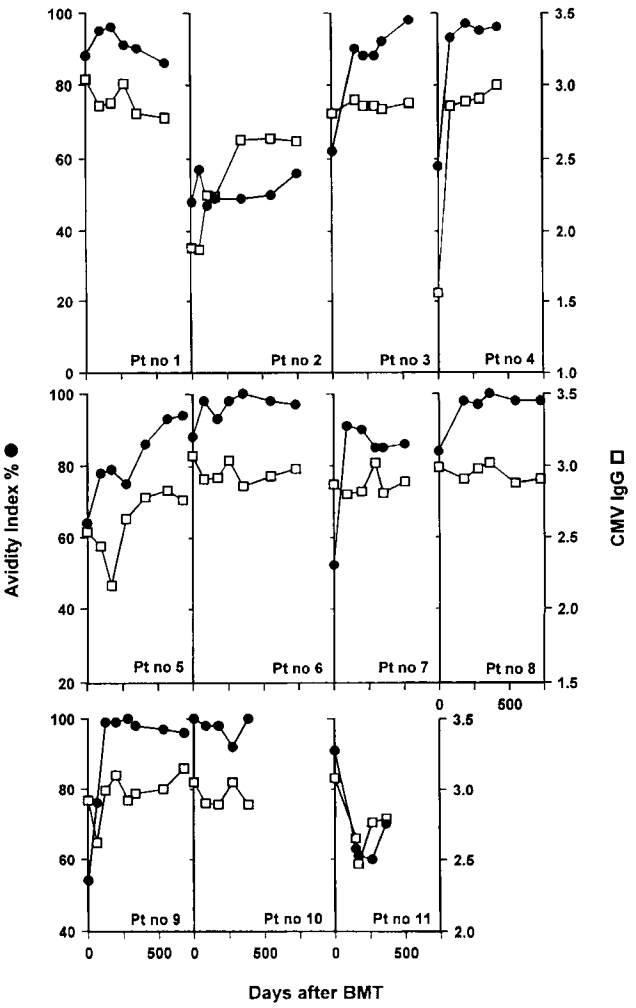

Fig. 1. Temporal changes in CMV IgG antibody and avidity after bone marrow transplant from a CMV seropositive donor to a CMV seropositive patient.

rise in IgG but no preceding drop and in patients 14 and 19 the antibody levels were not quantifiable (OD_{550} greater than 2.0). Overall in this group of patients with seronegative donors, antibody levels were lower after transplant than in the group with seropositive donors; thus the minimum OD_{550} dropped to below 2.0 in 5 cases and in 3 cases to below 1.5. However, no patient became seronegative; this finding was confirmed by the CMV latex test (data not shown).

The Effect of Donor CMV Antibody Status on Patients' Antibody Avidity Index After Transplant

Comparison of Figures 1 and 2, which in addition to qualitative data for CMV IgG also show the temporal variation of antibody avidity indices for each patient, demonstrates that overall the avidity index remains high after transplant in those patients whose donors were CMV seropositive (Nos. 1–11), whereas it decreases and then rises again in those patients whose donors were

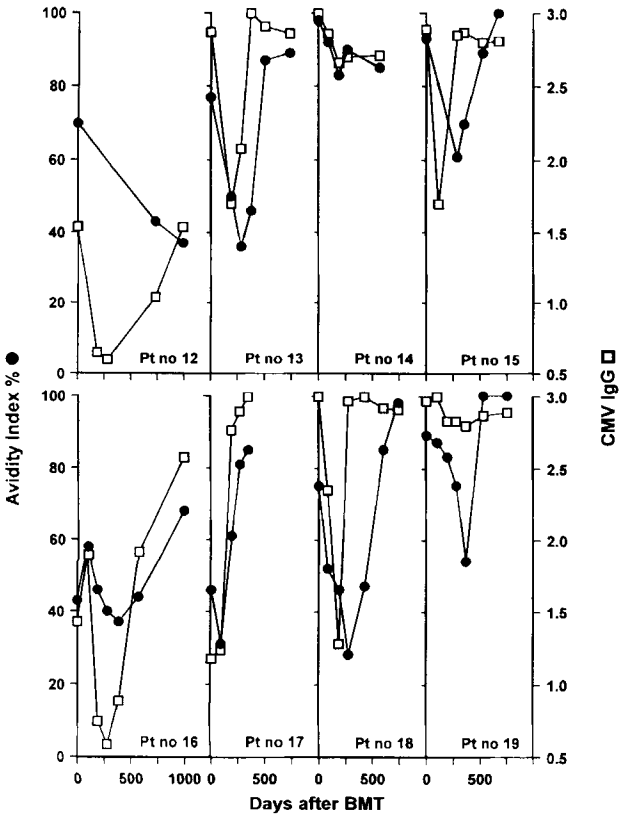

Fig. 2. Temporal changes in CMV IgG antibody and avidity after allogeneic bone marrow transplant from a CMV seronegative donor to a CMV seropositive patient.

CMV seronegative (Nos. 12–19). In this context it should be noted that the mean avidity indices prior to transplant were not significantly different for the two groups (72 [59–84] and 74 [57–91], respectively), and that these two means were likewise not significantly different from the mean antibody avidity index for the donors to patients 1–11, which was 73 [60–86].

Considering the patients whose donor was CMV seropositive in more detail (Fig. 1) the avidity index is high and tends to remain stable (nos. 1, 2, 6 and 10) or else to rise very soon after transplant (Nos. 3–5 and 7–9); patient no. 11 was anomalous showing a significant drop and then a slight rise in antibody avidity. Overall in this group of patients antibody avidity indices were between 80 and 100% after transplant except for patient 2 who showed stable values of approximately 50%.

In contrast, for patients whose donor was CMV seronegative (Fig. 2), there is a decrease in avidity index in each case immediately after transplant; the decrease was most marked in five patients (Nos. 12, 13, 15, 18, and 19) in 3 of whom the index dropped to below 40%. This decrease was usually followed by a steady increase in antibody avidity as seen in patients nos. 13, 15, 16, 17, 18, and 19 in whom the lowest avidity index occurred between about 250 and 500 days after transplant. This pattern is very similar to that described above for changes in amount of CMV IgG but it should be noted that antibody rises usually precede the corresponding rises in antibody avidity index by approximately 100 days (patients 13, 15, 16, and 18; Fig. 2). A late rise in avidity index was not seen at all in 2 patients; in patient 12 the index was at its lowest point, i.e., 37%, in the last serum available 993 days after transplant, and in patient 14 there was only a slight decrease in avidity index from 98% to 85% over 563 days.

Finally, the lowest avidity index for each patient after transplant was noted. The mean value for patients 1–11 with seropositive donors was 81 [71–91]. This was significantly higher than the value of 46 [30–61] for patients 12–19 with seronegative donors ($P < 0.001$).

DISCUSSION

The CMV IgG avidity ELISA used for this study had already been validated in a previous study of solid organ transplant patients [Lutz et al., 1994]. In that study avidity indices from well characterised sera from patients with either primary or long-term CMV infection were used to define a low avidity index as less than 35% whereas a high avidity index was greater than or equal to 60%. These figures are in good agreement with previous estimates for low and high avidity which were less than 30%, and greater than 50%, respectively [Hedman and Rousseau, 1989]. Turning to the present study, the CMV IgG avidity index observed before transplant when there was no reason to suspect primary CMV infection, was lower than 60% in several cases being 48%, 58%, 52%, 54%, 43%, and 46% for patients 2, 4, 7, 9, 16, and 17, respectively (Figs. 1, 2); a similar range of avidity indices was found for the CMV seropositive donors with 3 out of 11 giving values of 47%, 47%, and 58%, respectively. It was noted that lower avidity indices tended to occur in sera that had been stored at −20°C for several years as compared to recently collected sera (data not shown).

Lutz et al. [1994] had shown previously that the CMV IgG avidity test was robust and reliable over a wide range of antibody concentrations. The present results further confirm this observation since in patients whose donor was CMV seronegative (Nos. 12–19, Fig. 2) increases in CMV IgG usually preceded the corresponding rise in antibody avidity index by approximately 100 days, i.e., the avidity index is largely independent of antibody concentration. However, notwithstanding this, avidity indices for high avidity sera do increase slightly with antibody concentration where antibody levels are very high [Hedman and Seppälä, 1988; Lutz et al. unpublished data] as seen for patients 3–5 and 7–9 in Figure 1.

Turning to the evidence for transfer of humoral immunity from donor to recipient, CMV seropositive bone marrow transplant patients whose donors were CMV seropositive showed increasing levels of CMV antibody (Table 1) of high avidity (Fig. 1) early after transplant. These observations strongly support the concept of a donor-derived secondary response to CMV mounted by specific donor T and B memory cells as suggested by others in previous short term studies [Boland et al., 1992; Roy et al., 1993]. However, in our longer term

study it was also possible to derive clear evidence for a delayed primary antibody response to CMV in those CMV seropositive recipients whose donor was CMV seronegative. Overall there was an initial decrease in antibody levels after transplant (Table I, Fig. 2) followed by a rise (Fig. 2) which was followed by a similar fall and subsequent rise in antibody avidity (Fig. 2).

Looking at Figure 2 in detail it is clear that CMV IgG levels decreased soon after transplant in every patient but one, albeit at different times and rates, although no patient became seronegative. Such persistence of recipient antibody is in keeping with the prior observations of Wimperis et al. [1986], who showed that CMV-seropositive recipients continue to produce CMV antibody regardless of the serological status of the donor; this phenomenon is most probably explained by the persistence of recipient plasma cells resistant to both radiotherapy and chemotherapy that disappear gradually by attrition. In the present study, the initial decline in antibody in those patients with seronegative donors reached the lowest level within 250 days of transplant in the majority of cases and was followed by a marked increase (Nos. 12, 13, 15, 16 and 18), strongly suggesting that a delayed primary response to CMV occurred as virgin donor lymphocytes achieved immunocompetence and responded to CMV antigen.

As regards the changes in avidity index, in no case did the minimum avidity index fall within the range defined as low avidity [Lutz et al., 1994], although it did fall to below 40% in 4 cases. This is not surprising since the primary response is clearly initiated whilst persisting host B cells are still secreting some high avidity antibody, and the measured avidity index is thus a reflection of both low and high avidity antibodies. It is important to note that rises in antibody usually preceded the corresponding rise in avidity index by approximately 100 days—i.e., the primary response starts with low avidity antibody which matures to high avidity. Some variation from this pattern of dual antibody and avidity changes occurred in individual patients as would be expected in a population with varying rates of engraftment because of graft-versus-host disease, and subject to immunosuppressive therapy. However, it is clear that in general the primary response occurs at about 250 days after transplant and that maturation from low to high avidity takes between 100 to 200 days.

In conclusion, it was demonstrated by means of an antibody avidity test, that a delayed primary response to CMV occurs in those CMV seropositive recipients whose donors were CMV seronegative. The results also confirm previous evidence that humoral immunity may be transferred to CMV seropositive recipients from a seropositive donor. Thus, measurement of antibody avidity in this group of patients has added significantly to the understanding of the reconstitution of the humoral immune response to CMV after bone marrow transplantation.

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